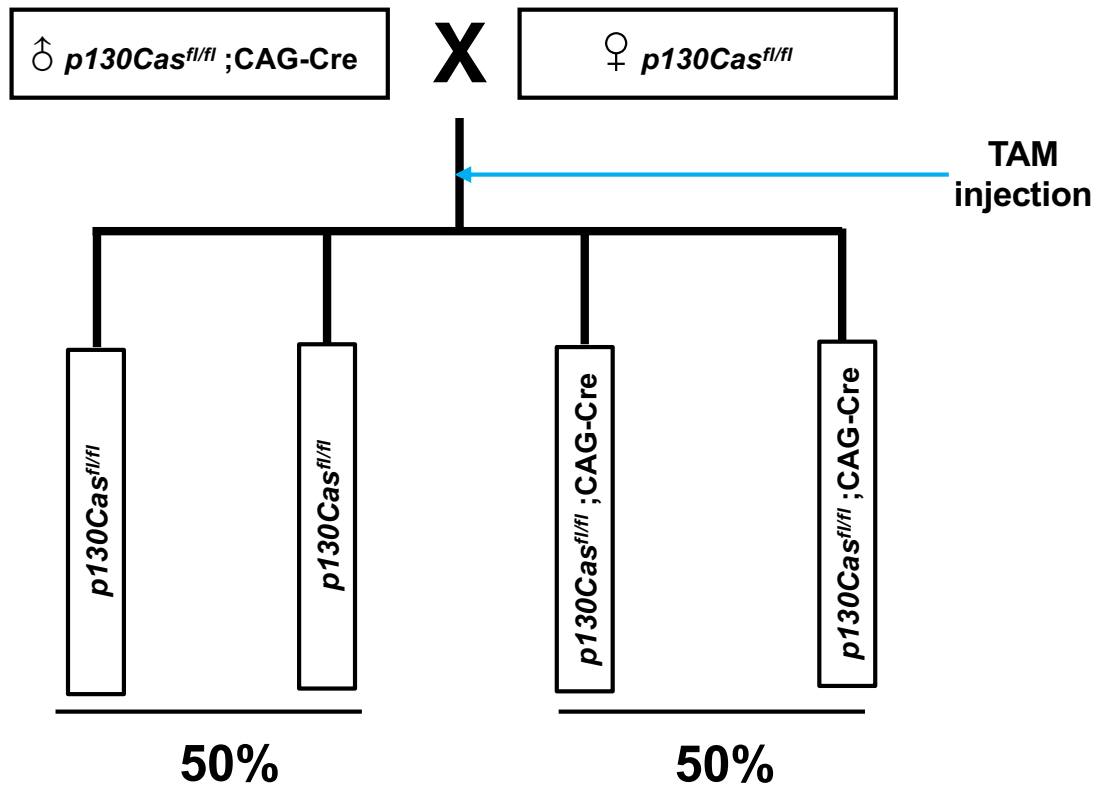
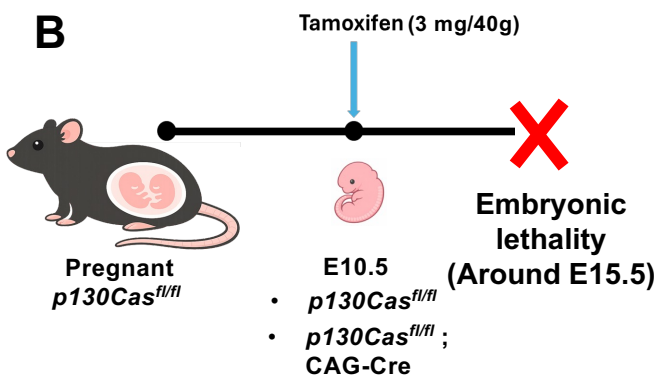


**A**



**B**



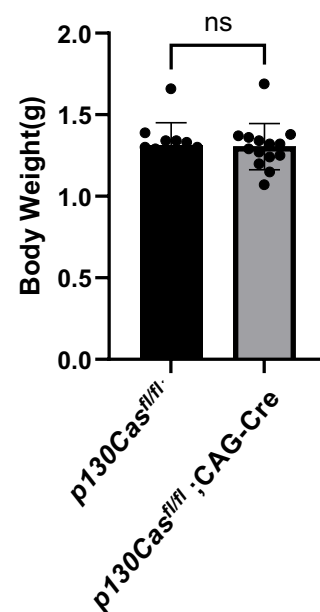
**C**

|                            | E10.5 | E11.5 |
|----------------------------|-------|-------|
| $p130Cas^{fl/fl}$          | 100%  | 100%  |
| $p130Cas^{fl/fl}; CAG-Cre$ | 0%    | 100%  |

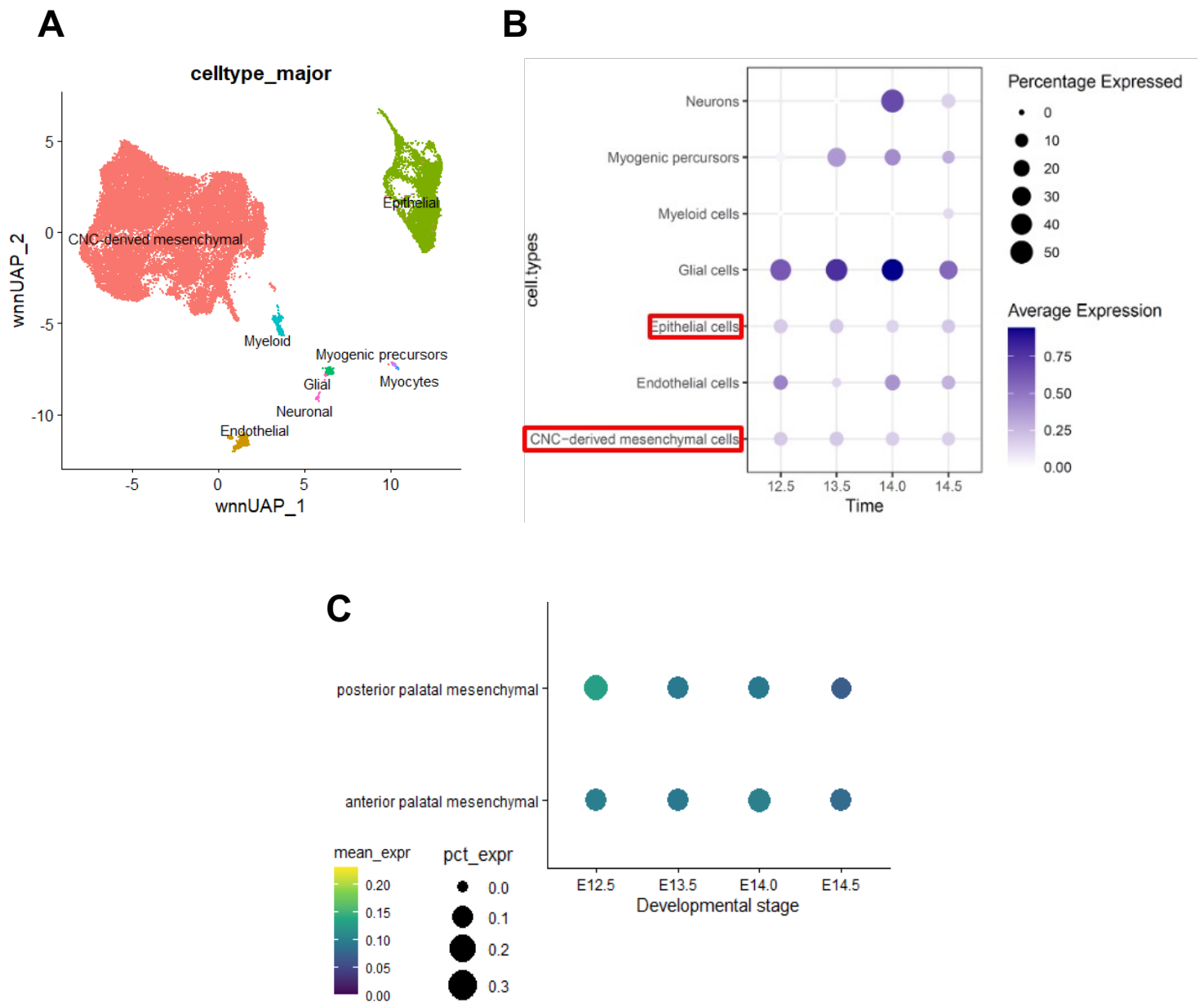
**D**



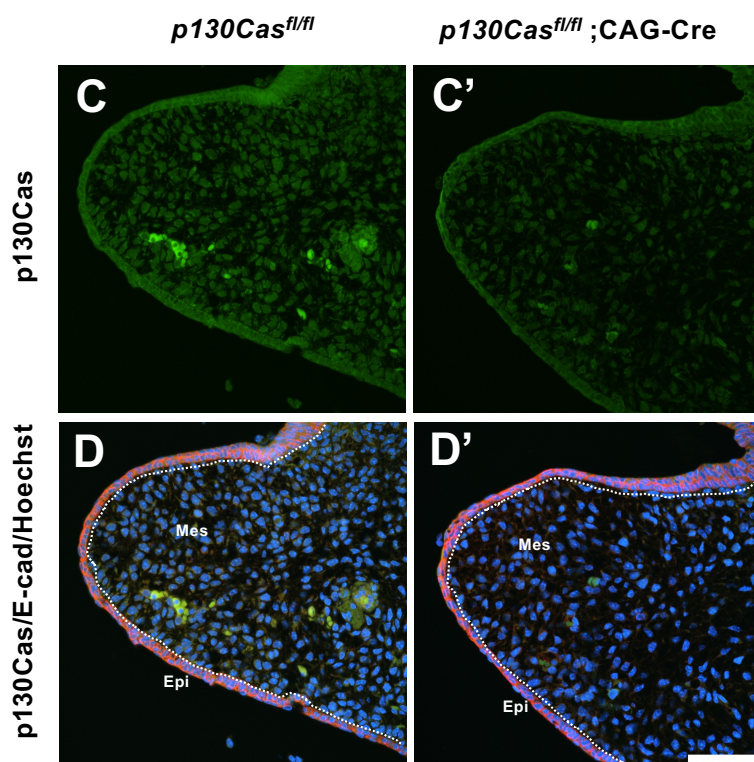
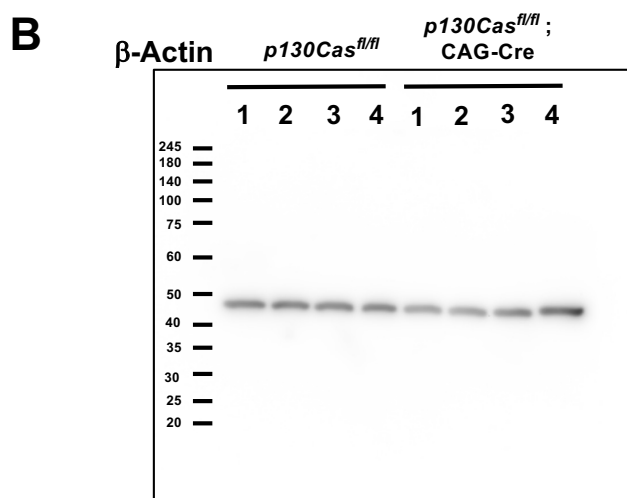
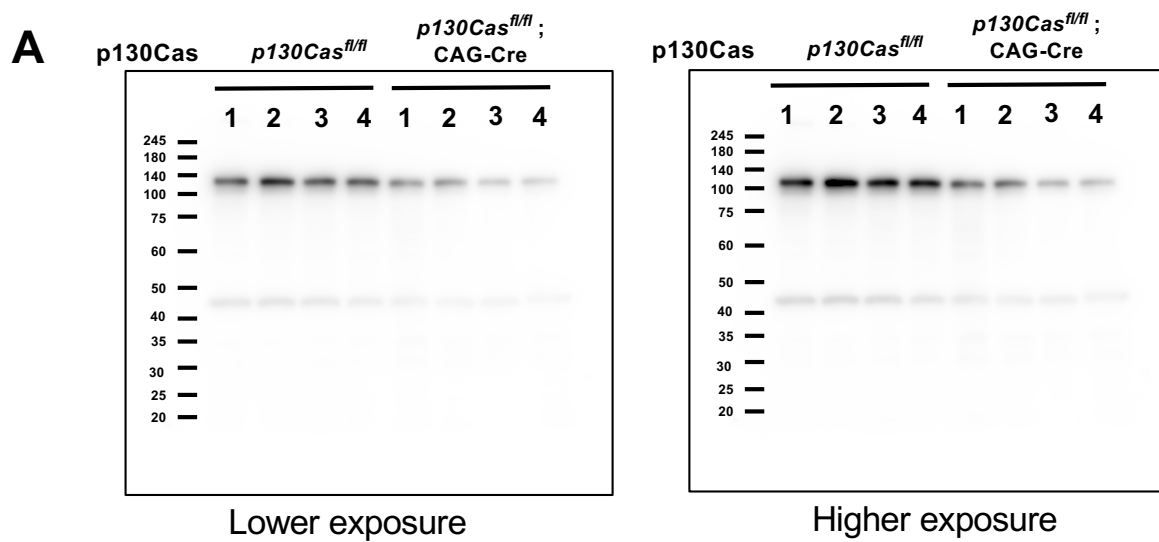
**E**



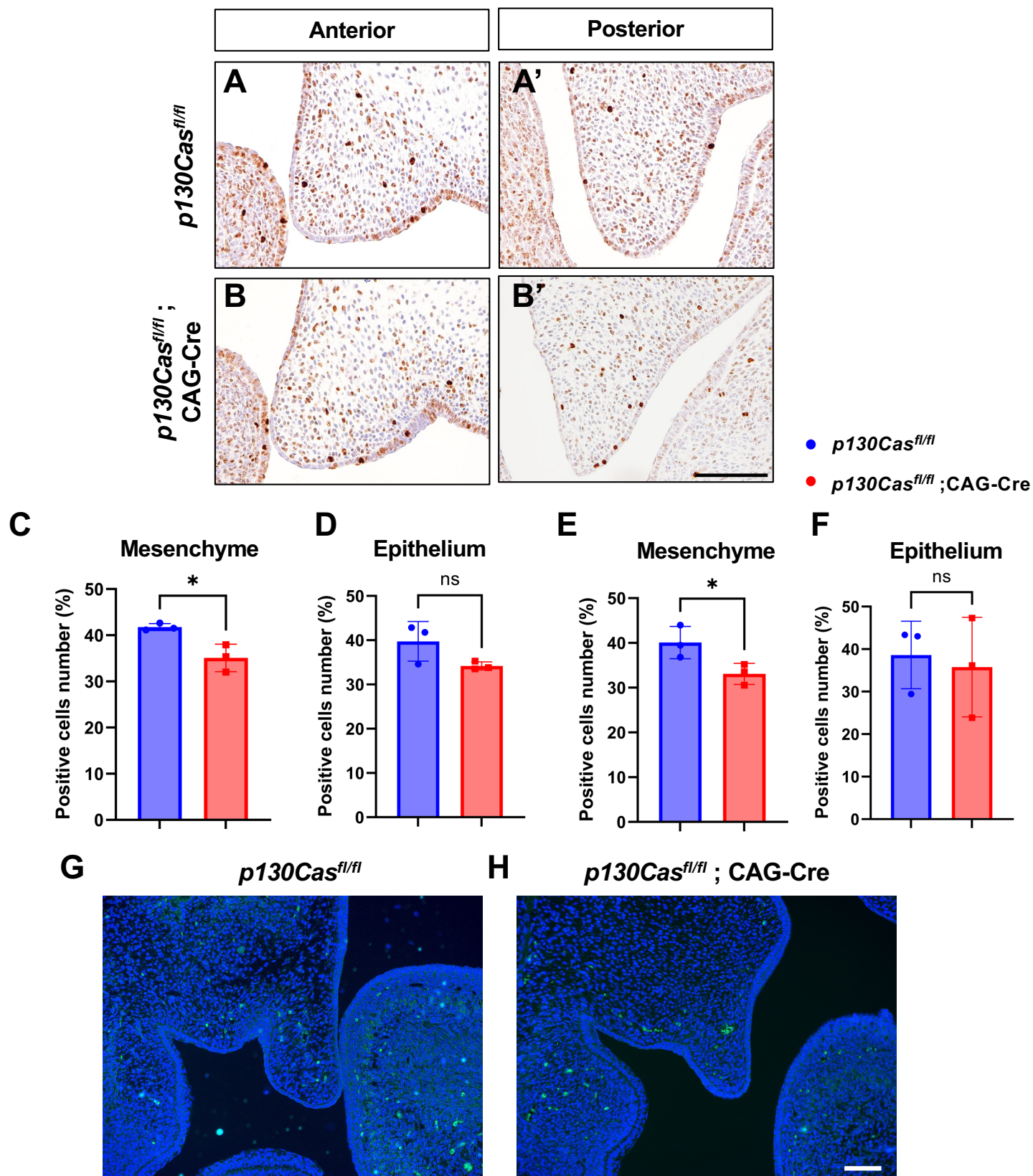
**Supplementary figure 1.** Generation of embryonic stage-specific p130Cas conditional knockout mice. **(A)** Breeding strategy showing the parental genotypes and expected offspring genotypes. **(B)** Schematic overview of the experiment design for time-dependent p130Cas knockout mice embryos. Tamoxifen injection at E10.5 leads to embryonic lethality. **(C)** Embryonic viability of *p130Cas<sup>fl/fl</sup>* and *p130Cas<sup>fl/fl</sup>; CAG-Cre* embryos following tamoxifen administration at E10.5 and E11.5. Percentages indicate the proportion of viable embryos recovered at E18.5 for each genotype. **(D)** Gross appearance of *p130Cas<sup>fl/fl</sup>* and *p130Cas<sup>fl/fl</sup>; CAG-Cre* embryos (E11.5 injection) at E18.5. **(E)** Body weight of *p130Cas<sup>fl/fl</sup>* (n= 12) and *p130Cas<sup>fl/fl</sup>; CAG-Cre* (n= 14) embryos at E18.5. Data show the means  $\pm$  SEM, N.S., no significance.



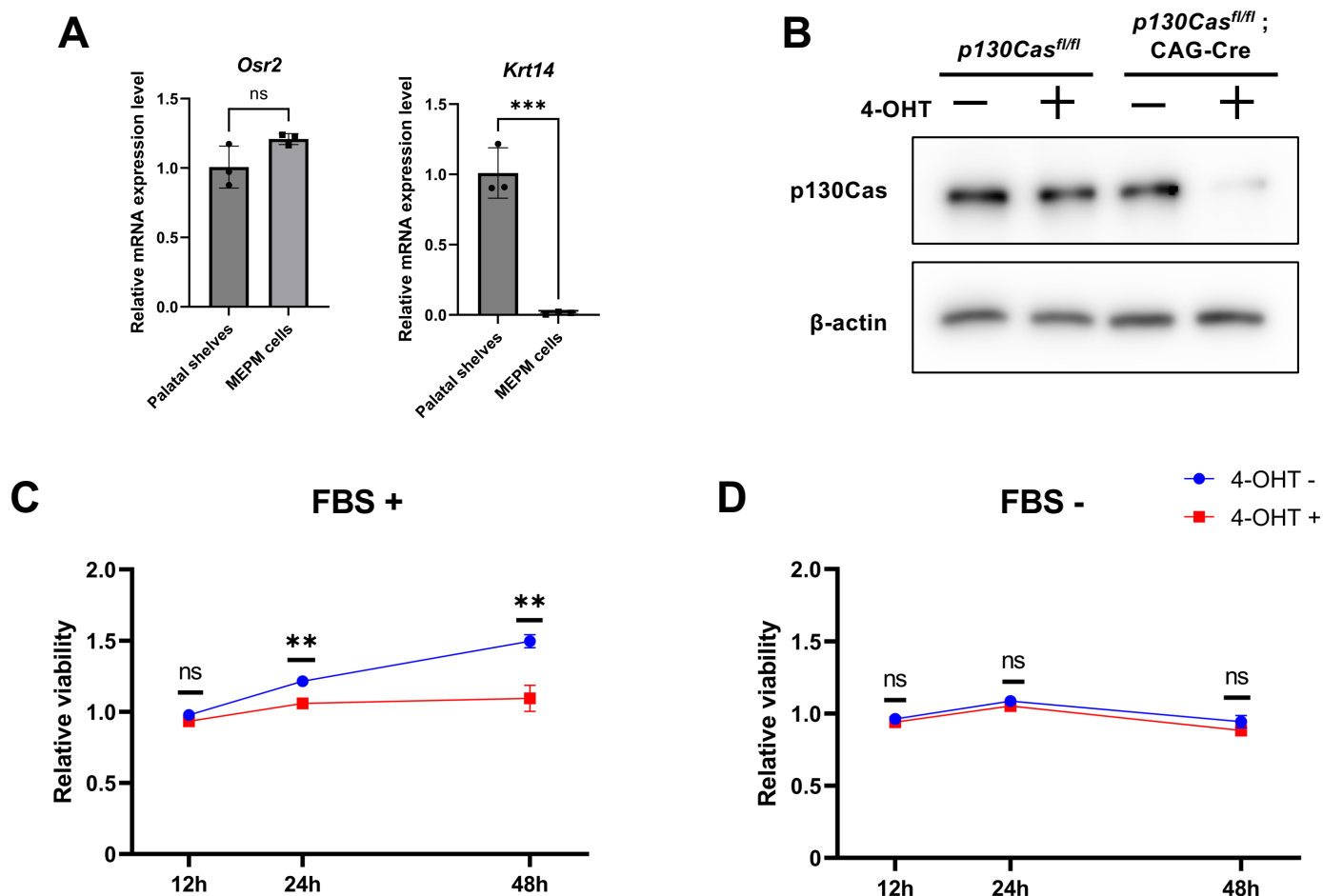
**Supplementary figure 2. (A)** Analysis of published single cell RNA (scRNA)-seq data (GSE218576) indicate that eight distinct cell types were defined during palatogenesis, including cranial neural crest cell (CNC)-derived mesenchymal (*Prrx1*), epithelial (*Krt14*), endothelial (*Cdh5*, *Cldn5*), myeloid (*Lyz2*), glial (*Plp1*, *Sox10*), myogenic precursor (*Myf5*), and neuronal cells (*Tubb3*, *Stmn2*), and myocytes (*Myh7*). **(B)** *p130Cas* expression in multiple cell types from E12.5 to E14.5. **(C)** *p130Cas* expression level along the anterior-posterior (A–P) axis.



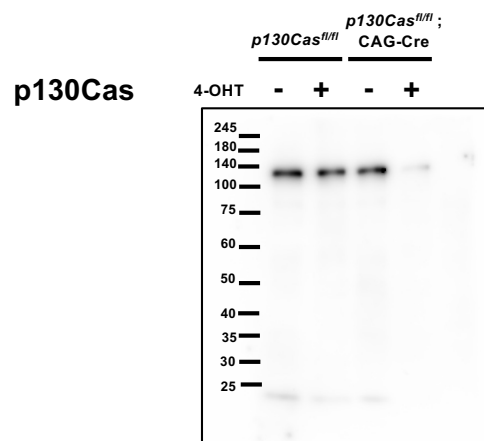
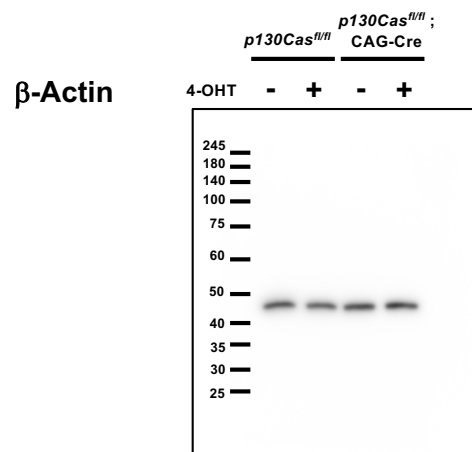
**Supplementary figure 3. (A and B)** Full-length uncropped immunoblot images corresponding to Fig. 2A. **(C-D')** Representative images for immunofluorescence staining of palate from E14.5 *p130Cas<sup>fl/fl</sup>* and *p130Cas<sup>fl/fl</sup>; CAG-Cre* mice embryos using anti-p130Cas and anti-E-cadherin antibody. A dotted line indicates the border between epithelial and mesenchymal tissues. Scale bars = 100  $\mu$ m. Epi: epithelium, Mes: mesenchyme. Representative images from three independent embryos per group are shown (n = 3). Scale = 100  $\mu$ m.



**Supplementary figure 4.** (A-B') Representative images for IHC staining of anterior and posterior palate from E13.5 *p130Cas<sup>fl/fl</sup>* and *p130Cas<sup>fl/fl</sup>; CAG-Cre* embryos using anti-Ki67 antibody. (C-F) Quantification of Ki67-positive cells in mesenchymal and epithelial region area in A to B'. *p130Cas<sup>fl/fl</sup>* (n = 3) and *p130Cas<sup>fl/fl</sup>; CAG-Cre* (n = 3) embryos. (G-H) Representative images for TUNEL staining of palate from E13.5 *p130Cas<sup>fl/fl</sup>* and *p130Cas<sup>fl/fl</sup>; CAG-Cre* embryos. nuclei were counterstained with Hoechst 33342. Scale = 100  $\mu$ m. Data show the means  $\pm$  standard error of mean, \* $P < 0.05$ , \*\* $P < 0.01$  versus the corresponding *p130Cas<sup>fl/fl</sup>* value.



**Supplementary figure 5.** (A) Quantitative real-time PCR analysis of relative mRNA abundance in isolated MEPM cells (n = 3) and palatal shelves tissue (n = 3) for mesenchymal cell marker (*Osr2*) and epithelial cell marker (*Krt14*). (B) MEPM cells isolated from *p130Cas<sup>fl/fl</sup>* and *p130Cas<sup>fl/fl</sup> ; CAG-Cre* embryos were treated with 4-hydroxytamoxifen (4-OHT) to induce p130Cas deletion. After 48 hours of treatment, proteins were extracted from cell lysates and analyzed by SDS-PAGE and immunoblotting using the indicated antibodies. (C) After 4-OHT treatment, MEPM cells were seeded in 96-well plate and cultured in medium with 10% FBS. WST-8 assay was performed at 12 h, 24 h and 48 h after cells were completely attached on the plate. Relative viability was normalized to the absorbance value at 12 h (n = 3 per group). (D) WST-8 assay was performed at 12 h, 24 h and 48 h in the absence of serum after starvation. Relative viability was normalized to the absorbance value at 12 h (n = 3 per group).

**A****B**

**Supplementary figure 6. (A and B)** Full-length uncropped immunoblot images corresponding to supplementary fig. 5B.